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13. ABSTRACT ***(Maximum 200 Words)***

The purpose of this study is to investigate the effects of soy and probiotic consumption on estrogen and phytoestrogen metabolism. The methods include *in vitro* studies to determine the intestinal microflora responsible for phytoestrogen metablism, and a human feeding study in which 18 postmenopausal breast cancer survivors and 18 controls consume four different dietary supplements for six weeks each. The supplements are: 1) soy powder; 2) soy powder + probiotic; 3) milk power; 4) milk powder plus probiotic. Urine is collected for three days before the study begins and for three days at the end of each diet period, for evaluation of urinary estrogen and phytoestrogen metabolites. Food records are collected on the same days as the urines. Feces are collected before the study begins and at the end of each diet period, for evaluation of intestinal microflora profiles. At this point, in vitro studies have been completed. The results suggest that cell culture is not a good model for human intestinal metabolism. All subjects have been recruited into the human feeding study, urines have been collected and processed and fecal samples have been analyzed. Ten subjects have completed the study and all subjects will be done by June, 2001.

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INTRODUCTION

Although most research on dietary prevention of breast cancer in Asia focuses on soy consumption, the Asian diet also contains large quantities of bacteria (probiotics) in fermented foods. For this project, we hypothesize that consumption of probiotic with soy may enhance soy's cancer-preventive effects by shifting phytoestrogen metabolism in a beneficial direction. The purpose of this study is to investigate the effects of soy and probiotic consumption on estrogen and phytoestrogen metabolism. The specific aims are to identify the intestinal bacteria responsible for phytoestrogen metabolism, via *in vitro* studies, and to determine the independent and interactive effects of soy and probiotic consumption on estrogen and phytoestrogen metabolism in women who have had breast cancer and controls. First, *in vitro* studies will be performed to determine which intestinal microflora are responsible for production of the phytoestrogen metabolite equol, which has been associated with low risk of breast cancer. Next, a randomized crossover human study will be performed, in which 18 postmenopausal breast cancer survivors and 18 age-matched controls will consume 4 different dietary supplements for six weeks each, separated by 2-week washout periods. The supplements are: 1) soy powder; 2) soy powder + probiotic; 3) milk power; 4) milk powder plus probiotic. Urine will be collected for 3 days before the study begins, and for 3 days at the end of each diet period, for evaluation of urinary estrogen and phytoestrogen metabolites. To assure that energy and nutrient intakes do not differ between diet periods, food records will be collected on the same days as the urines. Feces will be collected once before the study begins, and once at the end of each washout and diet period, for evaluation of intestinal microflora profiles.

BODY

According to the original statement of work, the following tasks were to be performed during the first year of this project:

1. Characterize phytoestrogen metabolism by specific intestinal bacteria:

    - Perform *in vitro* studies to evaluate the profile of phytoestrogen metabolites produced by intestinal bacteria such as *Bifidobacterium longum* and *Lactobacillus acidophilus*, in the presence of phytoestrogens

    In vitro studies were performed using *Bifidobacterium* and *Lactobacillus* bacteria, in the presence of daidzein, genistein and soy protein powder. The concentrations of phytoestrogen and soy used approximated the concentrations of phytoestrogens found in human plasma after soy consumption. Bacteria and control samples were incubated with appropriate media containing the phytoestrogens or soy powder, for 0-72 hours, after which aliquots of media were analyzed for phytoestrogen content using GC-MS methodology. Of specific interest was equol concentration, since equol has been associated with lowered risk of breast cancer. Unfortunately, only trace amounts of equol and other phytoestrogens were found, suggesting that a purified culture system does not

mimic phytoestrogen metabolism in the human gut. It is possible that metabolism of phytoestrogens results from complex interactions among numerous intestinal bacteria and other endogenous factors such as pH and bacterial substrates including dietary fiber and carbohydrate. We are investigating other *in vitro* systems that may be more appropriate for studying intestinal microflora, such as a fecal fermentation system.

2. Determine the independent and interactive effects of soy and probiotic consumption on intestinal microflora, urinary phytoestrogens and estrogen metabolites in women who have had breast cancer and controls.

   - Recruit 18 women who have never had breast cancer
   - Perform randomized crossover feeding study with women who have never had breast cancer, perform fecal assays, process and store urines

   We have made the following changes in the organization and scope of the study as originally written:

   - Instead of studying the control women in the first year and the breast cancer survivors in the second and third years, we decided that it was better from a statistical and practical viewpoint to begin recruiting both groups of women during the first year. The advantages of this change are 1) comparison between the two groups is not affected by different recruiting years; and 2) we have more time to recruit breast cancer survivors, which we knew would be difficult due to our strict exclusionary criteria.

   - We modified the design of the study to add a control milk powder. The original design had three diet periods: soy powder alone, soy + probiotic, and probiotic alone. The modified design has four diet periods: soy powder alone, soy + probiotic, milk powder alone, and milk powder + probiotic. We believe that this has added considerable strength to the original design. We also added a placebo capsule to be taken during the soy alone and milk powder alone diet periods. Unfortunately, there was a problem with the placebo capsules, which were found to be contaminated with probiotic. We discontinued their use as soon as this was discovered, and diet periods during which placebo capsules had been taken were repeated.

   - The original plan was to use the results of the *in vitro* studies to choose the probiotic supplement. Since the *in vitro* studies were not able to determine the exact bacteria responsible for phytoestrogen metabolism, the commercial supplement used in our pilot study (DDS-plus, UAS Laboratories) was used.

   We have successfully stayed on schedule during the first year. Eighty-five women were interviewed, of which 61 women underwent health screen

evaluations for the study. Of those women, 53 have been enrolled in the study since it began in November, 1999. Fourteen women have dropped out of the study as a result of difficulties due to dietary restrictions (1), peri-menopausal status (1), unrelated medical issues (3), incompatible travel plans (4), and time conflicts (5). Thus, 39 subjects have remained in the study (20 controls and 19 breast cancer survivors). At this point 10 controls and two breast cancer survivors have completed the study. Of the 27 subjects remaining, 16 will finish by December, 2000 (10 controls and 6 survivors). The remaining 11 subjects (all breast cancer survivors) will be finished by June, 2001. Fecal samples have been analyzed for microflora profiles, and data have been entered into an Excel spreadsheet. Preliminary data analysis is underway, and an abstract of the fecal microflora data may be submitted to Experimental Biology '01 (abstract submission deadline is November 6, 2000). All urine samples have been collected, processed and stored as stated in the original proposal.

KEY RESEARCH ACCOMPLISHMENTS

- Successfully recruited the proposed number of subjects and carried out human feeding study
- Collected urine samples in human study, processed and stored urine
- Collected fecal samples in human study, evaluated fecal microflora

REPORTABLE OUTCOMES

None at this time

CONCLUSIONS

The human feeding study has been successfully carried out and all biological samples have been analyzed or stored as stated in the study design. At this point there are no reportable data from which to draw conclusions. We have learned the difficulty of recruiting breast cancer survivors, and have developed a summary of our recruiting methods in order to evaluate which are the best for this population. As a result of the lack of success of our *in vitro* model, we are discussing the possibility of trying a fecal fermentation model to replace the cell culture model used in the *in vitro* studies. By the end of the second year (September 14, 2001), all subjects will have completed the study, fecal data will be analyzed and submitted for publication, and urinary phytoestrogen and estrogen metabolite analysis will have begun. We will have no problem completing all tasks by the end of the grant period.

REFERENCES

None

APPENDICES

None